ADVANCED BIO-CATALYTIC MICROREACTOR WITH NANOPARTICLES FOR ENHANCING LIPID PRODUCTION IN MICROALGAE

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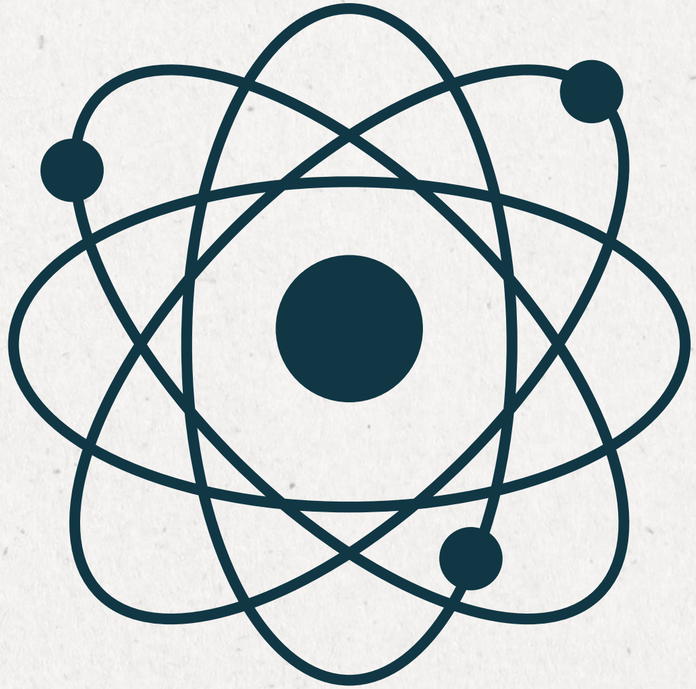
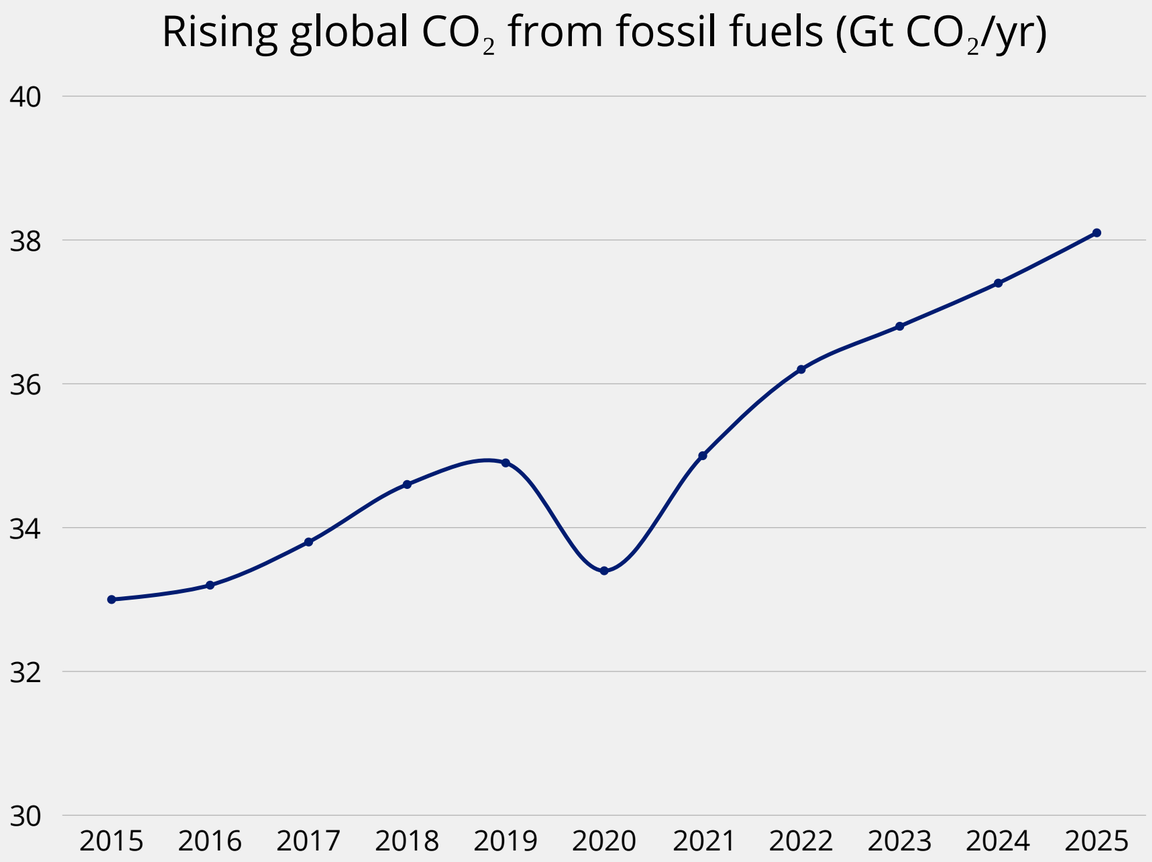


GLOBAL PROBLEM



Global Carbon Budget 2025 — CSIRO / Global Carbon Project (released at COP29, Baku, Azerbaijan)

2025 fossil fuel emissions

38.1 Gt

CO_2 projected this year alone

Atmospheric CO_2 concentration

425 ppm

+52% above pre-industrial levels

WHY MICROALGAE ?

- Rapid Growth
- Sustainable Cultivation
- High Lipid Potential
- CO_2 Fixation

"Microalgae are theoretically capable of supplying the world's entire transportation fuel demand using less than 3% of current global cropland — the challenge is unlocking their lipid productivity at scale."

THE GAP

01 Low Lipid Productivity

02 Lack of Process Control

03 Need for Process Optimization

OBJECTIVES

01

Nanoparticles Enhancement

02

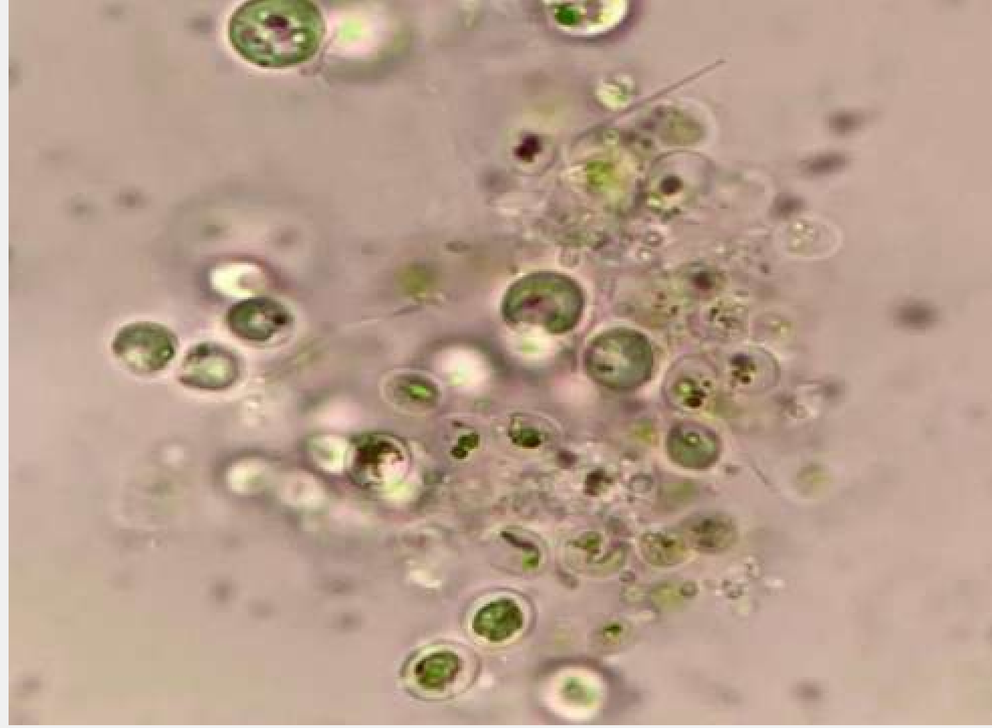
Microreactor Cultivation System

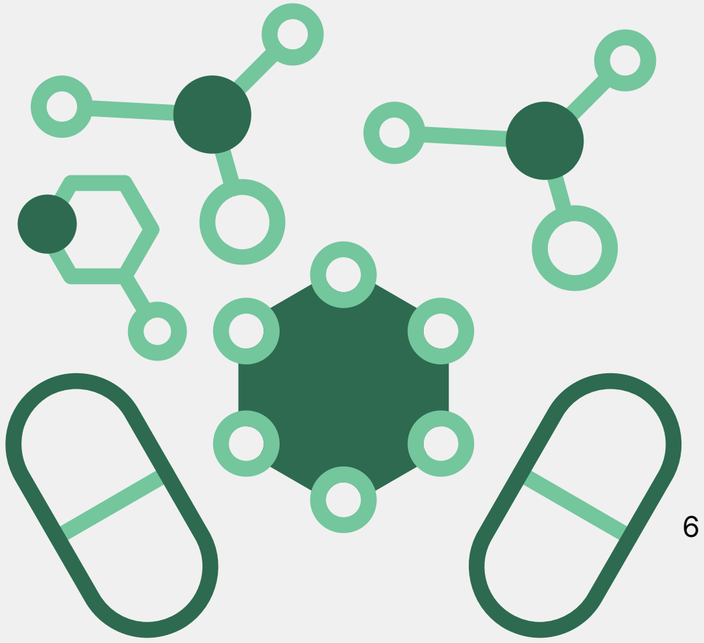
03

Process Optimization

LITERATURE REVIEW & THEORETICAL BACKGROUND

- Microalgae
- Nanoparticles
- Microreactor Systems

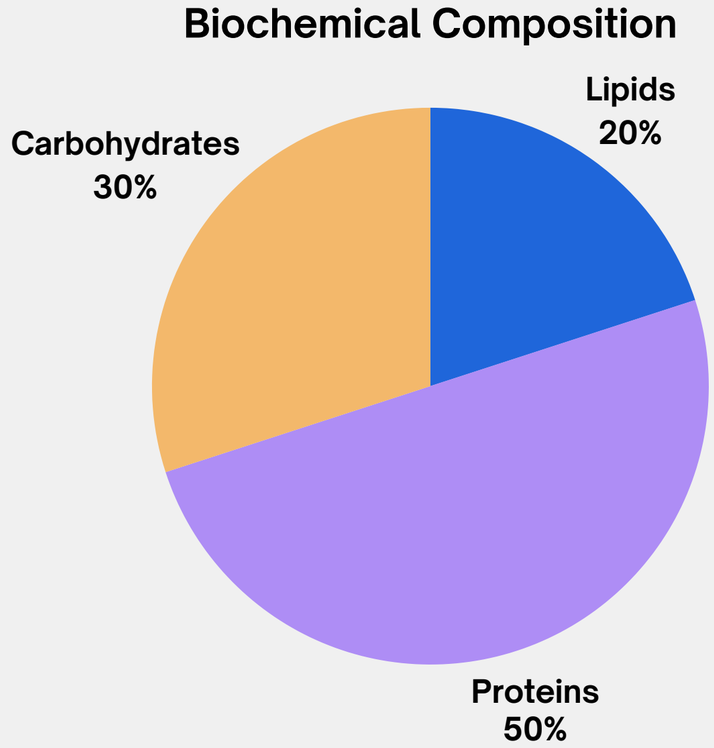




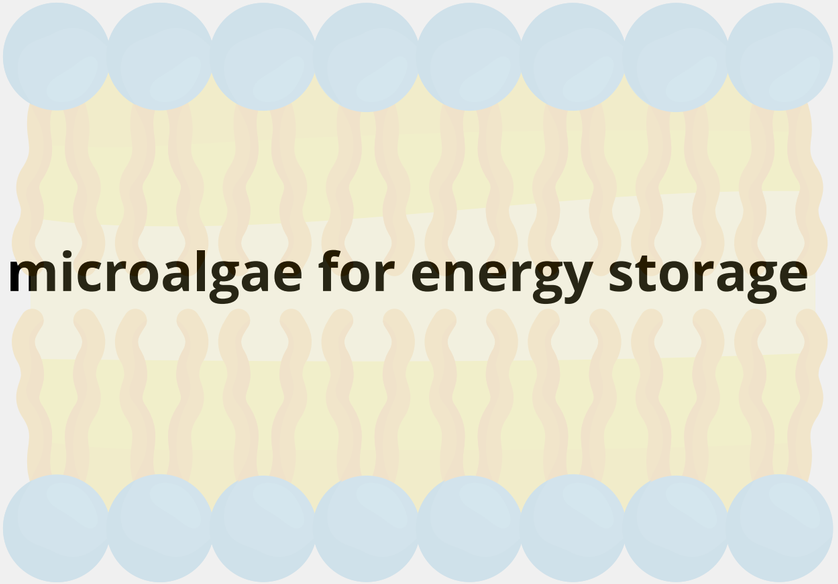
Microalgae

single-celled, photosynthetic microorganisms found in freshwater and marine environments

- ⚡ Rapid growth
- 🛢️ High lipid potential
- 🌍 CO_2 utilization
- 🌱 Renewable resource



Lipid Production in Microalgae



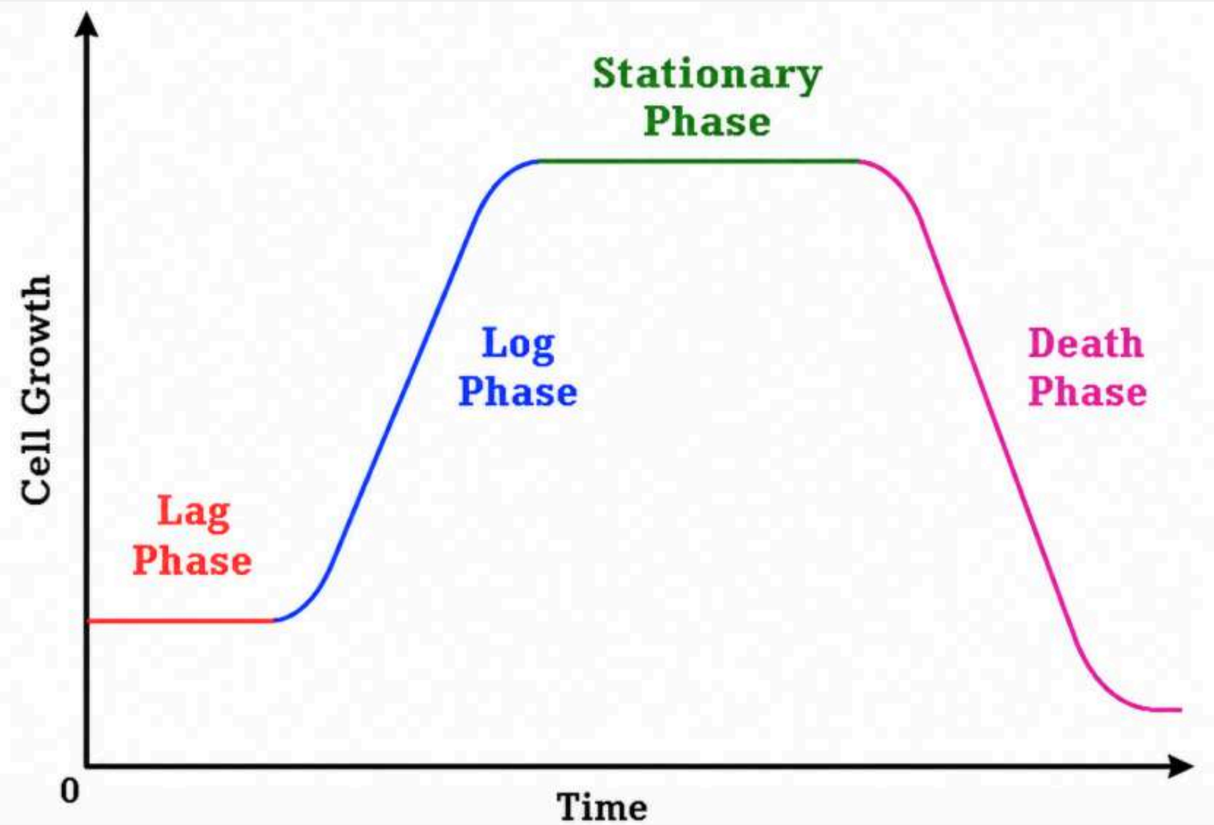
Lipids are hydrophobic organic compounds synthesized by microalgae for energy storage and cellular functions

- Neutral lipids (TAGs)
- Polar lipids
- Glycolipids
- Phospholipids

- 🛢️ Energy storage
- ⚡ High energy density
- 🧪 Industrial raw material
- 🌱 Renewable source

- 🛢️ Biodiesel
- 💊 Pharmaceuticals
- 🍱 Nutraceuticals
- 🧴 Cosmetics

Microalgae Growth Phases



Lag Phase

Cell adaptation

Exponential Phase (LOG)

Rapid cell division

Stationary Phase

Nutrient depletion & lipid accumulation

Death Phase

Cell death increases

- **lipid accumulation mainly increases during stationary phase**

Lipid Accumulation Mechanism

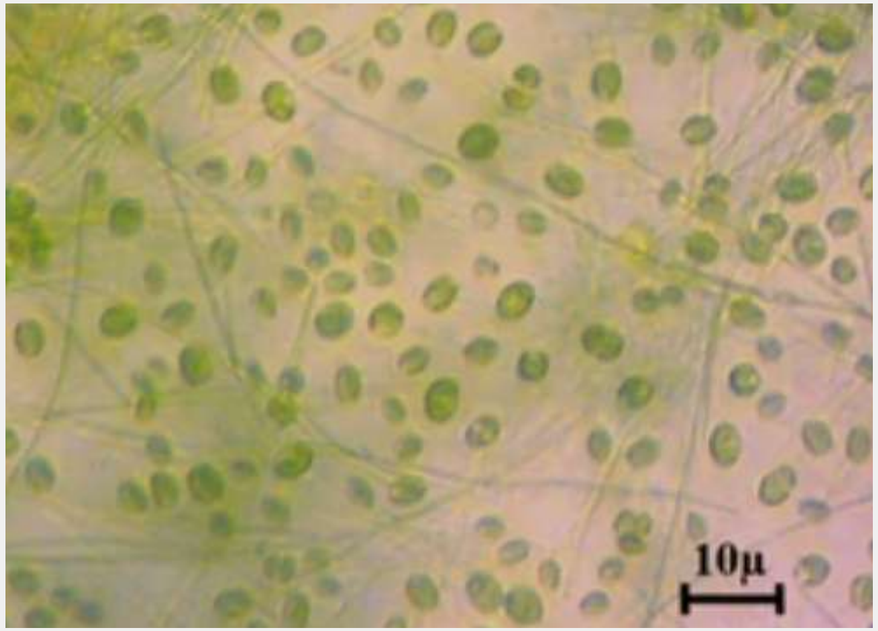
- Light Stress
- Nutrient Limitation
- Temperature Stress
- Nanoparticle Interaction

STRESS FACTORS →

Nutrient Limitation
↓
Reduced Cell Division
↓
Metabolic Shift
↓
TAG Accumulation
↓
Lipid Storage

- **Stress conditions redirect metabolism from biomass growth toward intracellular energy storage**

Picochlorum sp. Characteristics



- High salinity tolerance
- Small cell size & high surface-area-to-volume ratio
- Adaptable to environmental stress
- Strong lipid accumulation under stress conditions

- Its small cell size makes it highly suitable for microreactor systems

Small cell

⬆️ Mass transfer
⬆️ Nutrient transport
⬆️ Nanoparticle interaction

Nanoparticles in Microalgae Cultivation

- ⚡ Large surface-area-to-volume ratio
- 🌱 Enhanced nutrient interaction
- 🧪 Influence cellular metabolism
- ☀️ Improved light utilization
- 🛢️ Increased lipid biosynthesis

- Nanoparticles were used to enhance lipid accumulation and improve cultivation performance in *Picochlorum sp.*

Starch & Alginic Acid Nanoparticles

Property	Starch NP	Alginic Acid NP
Source	Natural polysaccharide	Brown algae-derived polysaccharide
Toxicity	Low	Low
Main Effect	Mild metabolic stimulation	Stronger cellular interaction
Lipid Enhancement	Moderate	Stronger lipid enhancement
Interaction with Cells	Moderate	Strong

Microreactor Systems

Parameter	Microreactor	Batch Reactor
Surface area to volume ratio	Very high	Low
Mass transfer	Enhanced	Limited
Light distribution	More uniform light exposure	Non-uniform light penetration
Cultivation performance	Improved cell interaction	Reduced interaction efficiency

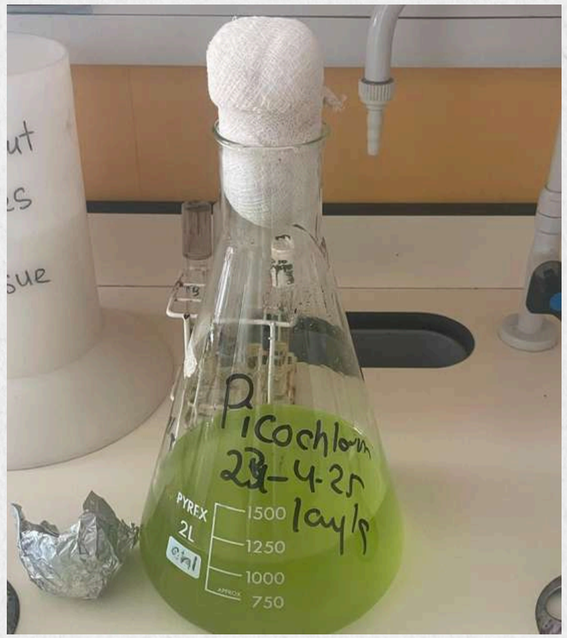
PRIMARY BATCH EXPERIMENTS

Expermintal Procedure

1. Mixing 750 ml Fertilizer Sea Water with 250 ml Stock Culture, ratio 3:1

2. Adding F2 Media (nutrients source) to the culture , ratio 1 ml : 1 L

3. Cover the flasks with aluminum foil to maintain cultivation conditions



Primary Batch Experiments

Treatment Groups

Alginic acid nanoparticles, alginic acid bulk, starch nanoparticles, starch bulk, and control

Treatment Concentrations

100 ppm and 500 ppm

Cultivation Conditions

12 h light / 12 h dark photoperiod under similar environmental conditions

Experimental Duration

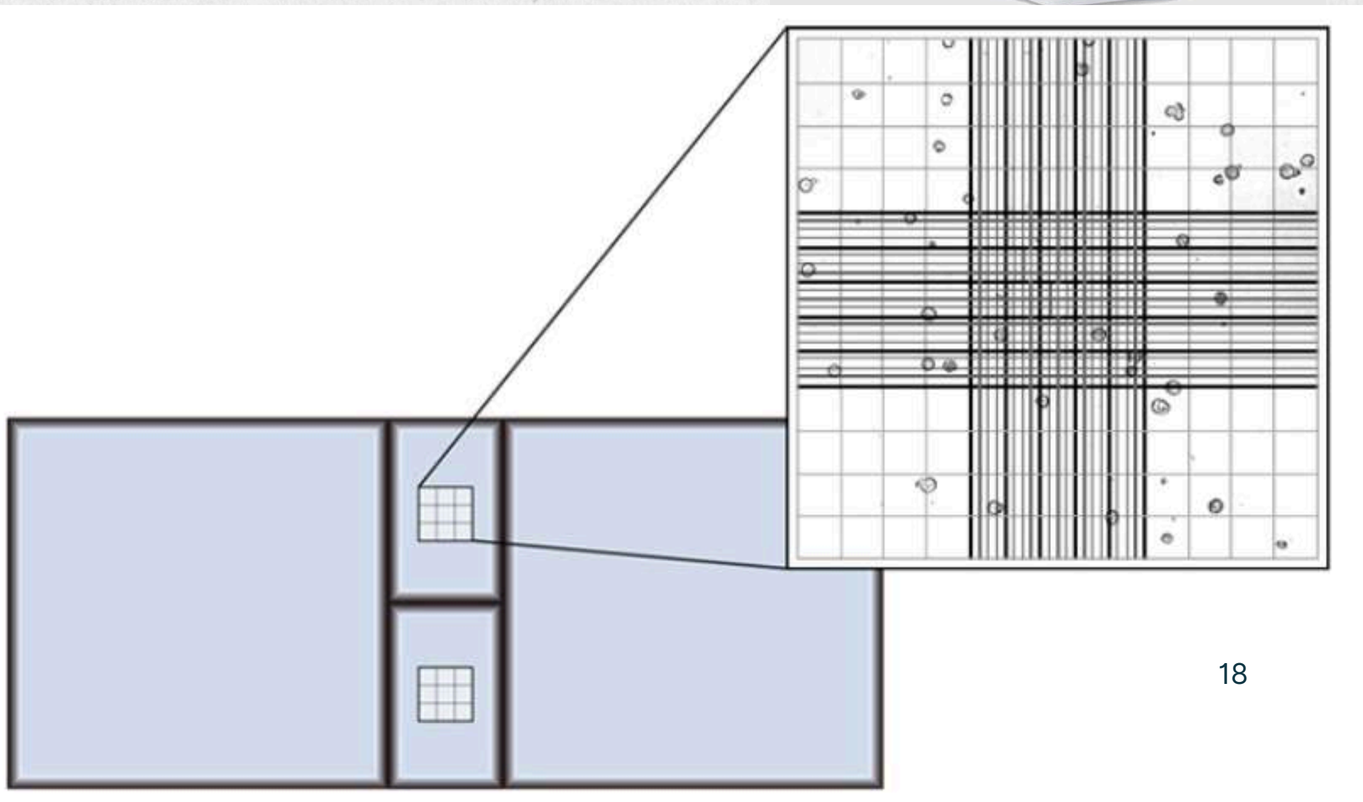
Cultivation period of 12 days

CELL CONCENTRATION MEASUREMENT

Hemocytometer Counting Method:

Cell Concentration (cells/ mL) = total cells counted × 50000 × dilution factor

- **Cell counting performed using hemocytometer chamber**
- **Microscopic counting used for growth monitoring**
- **Measurements conducted throughout cultivation period**



Lipid Extraction & Determination

Bligh & Dyer Extraction Method

Freeze-Dried Biomass

↓

Methanol + Chloroform + Saline Addition

↓

Mixing & Centrifugation

↓

Chloroform Layer Separation

↓

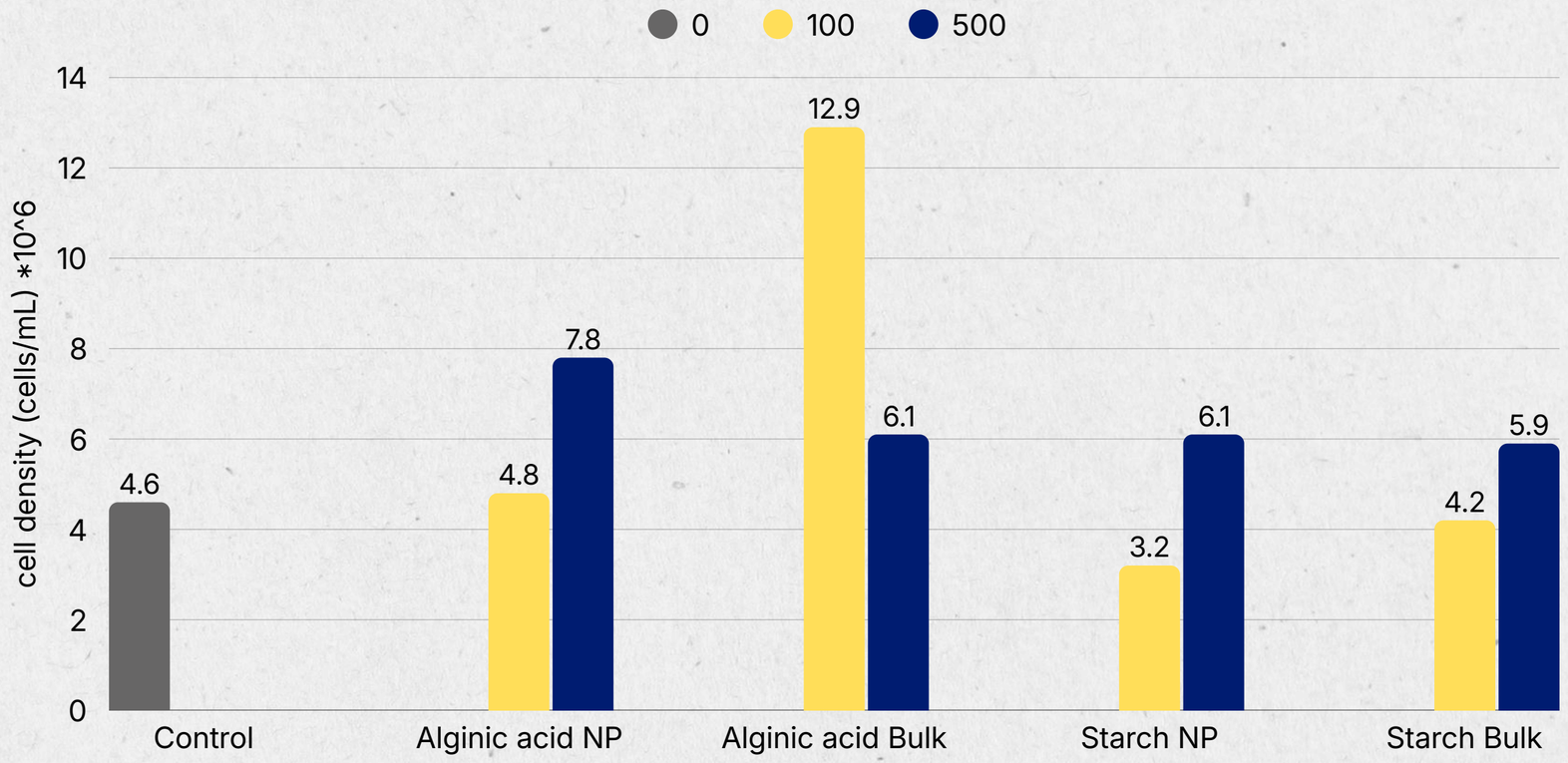
Nitrogen Gas Evaporation

↓

Lipid Weight Measurement

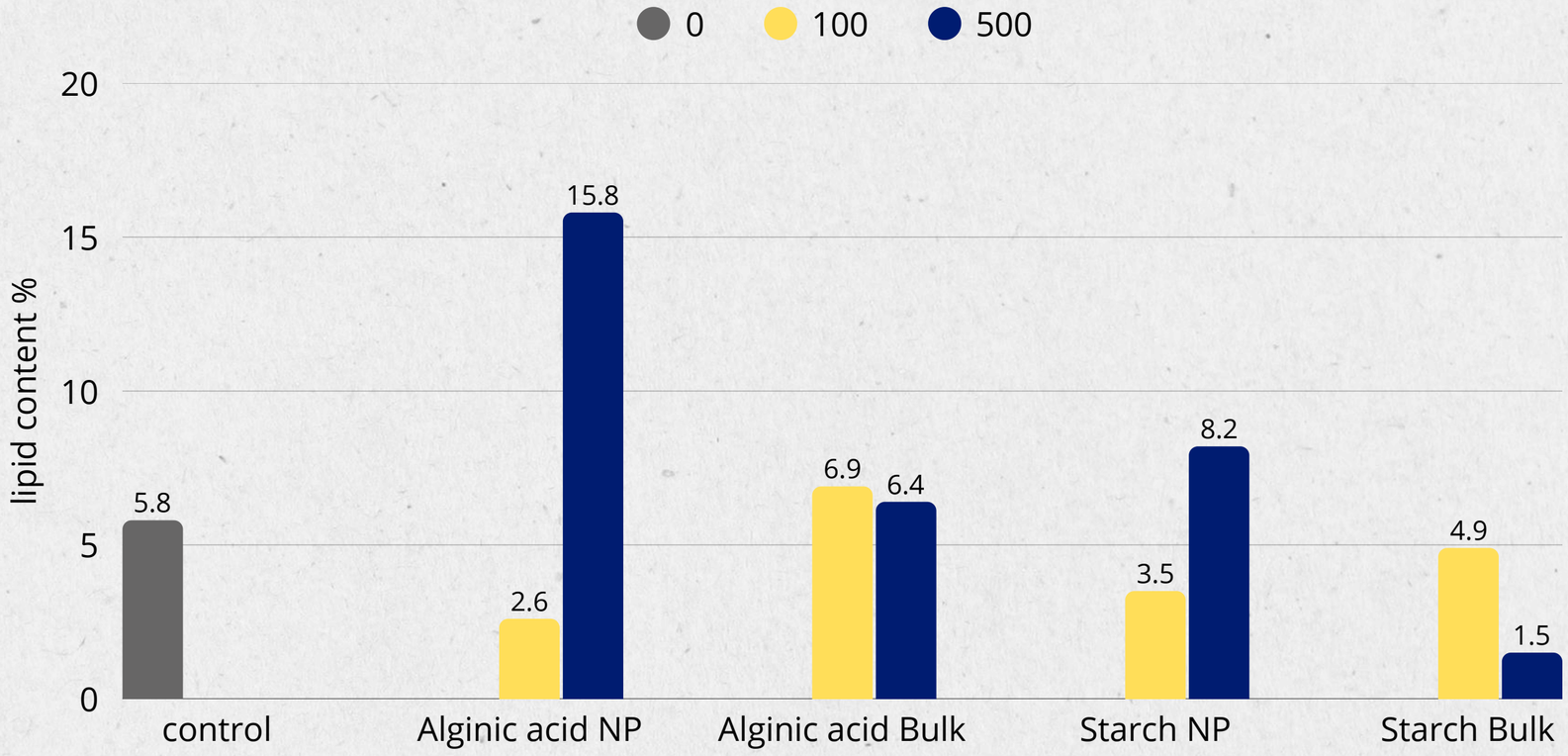
Lipid Content (%) =Weight of Lipid / Weight of Dry Biomass x 100

CELL CONCENTRATION RESULT



- **Highest cell growth: Alginic acid bulk, 100 ppm**
- **Starch treatments showed lower growth compared with alginic acid**

LIPID CONTENT RESULT

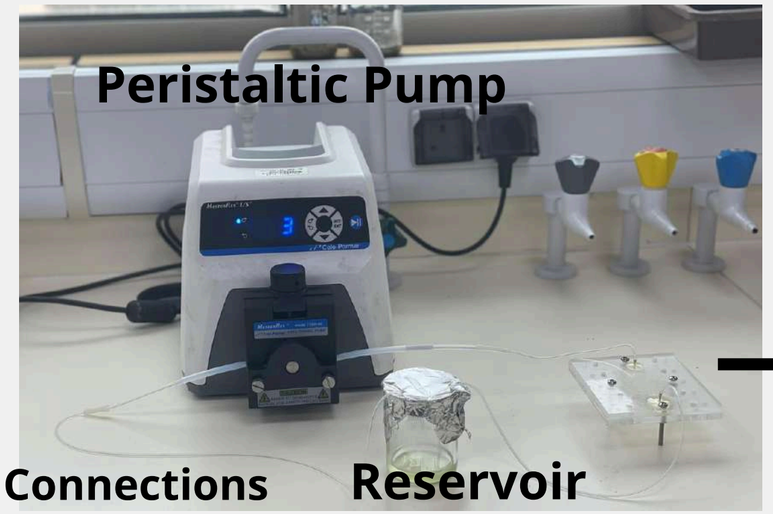


- **Highest lipid content: Alginic acid NP, 500 ppm = 15.8%**
- **Nanoparticles performed better than bulk additves**

BATCH VS MICROREACTOR COMPARISON

MICROREACTOR CULTIVATION SETUP

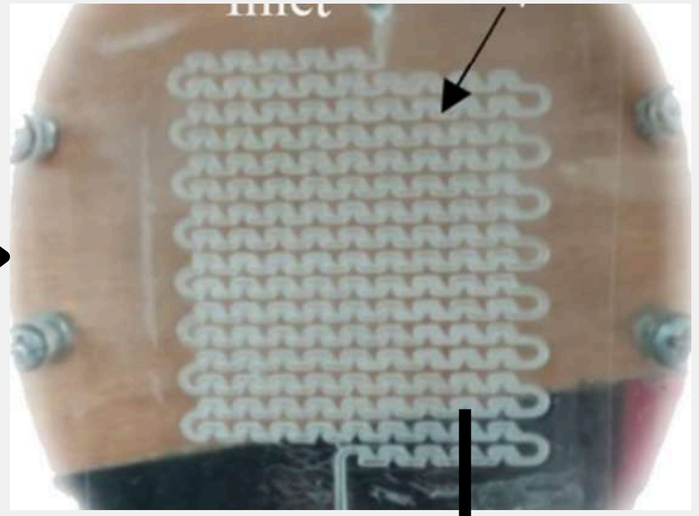
Peristaltic Pump

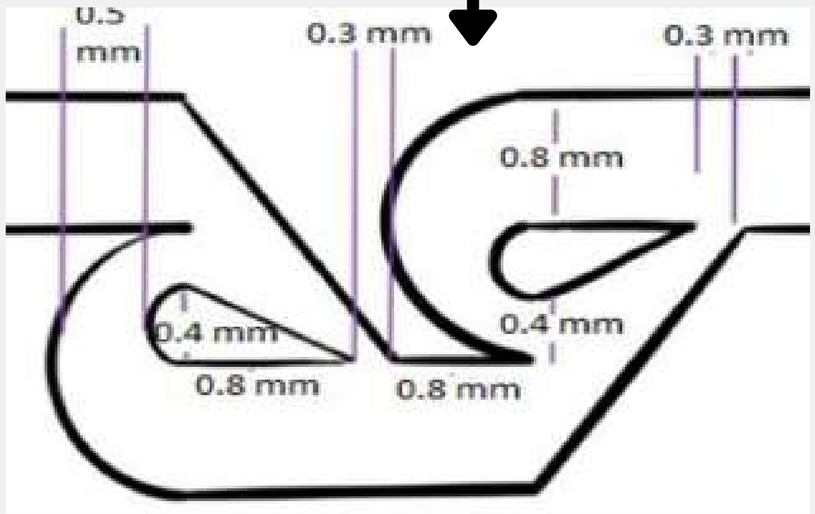


Tubing Connections

Reservoir

Tesla Microreactor





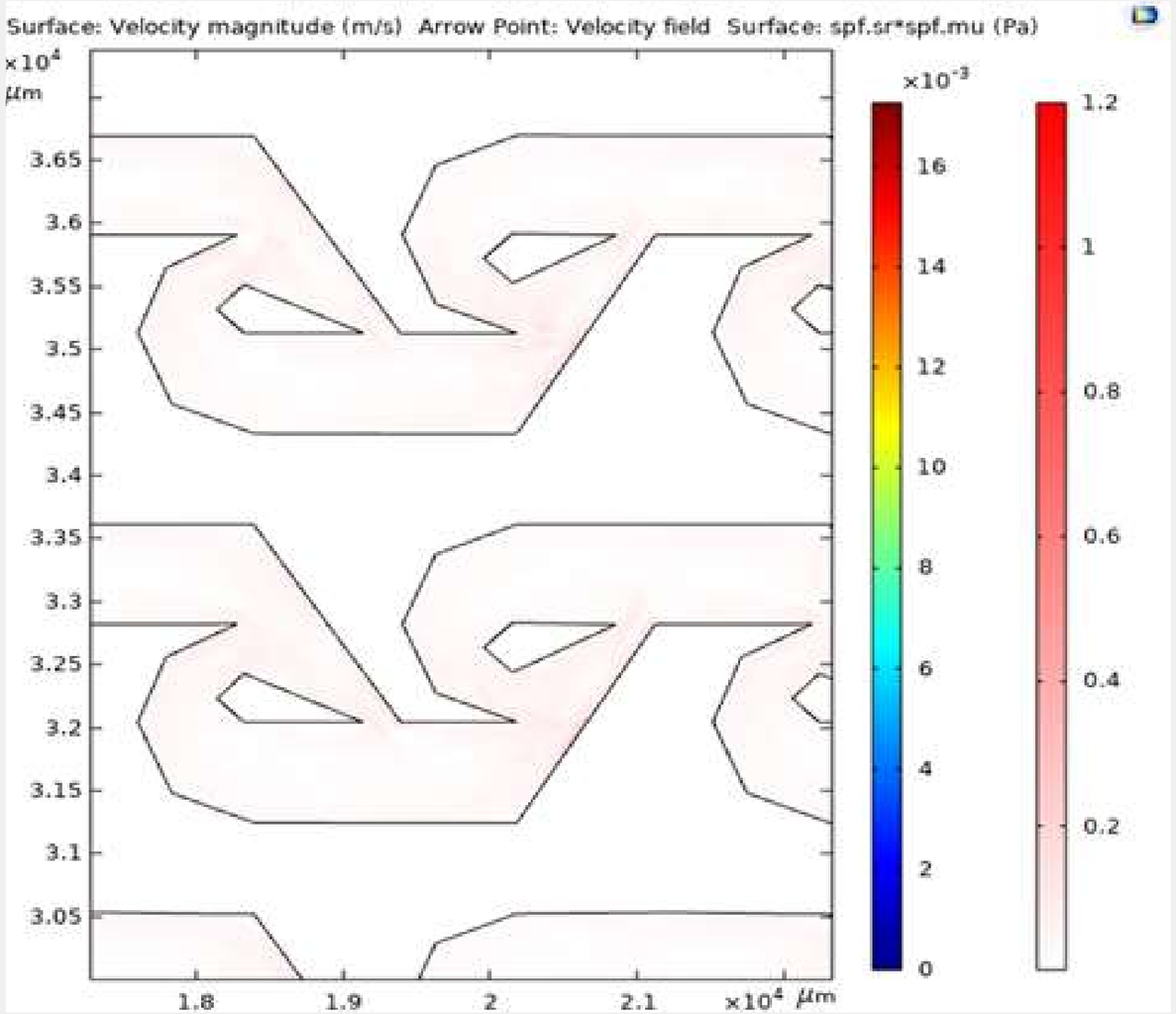
MICROREACTOR VS BATCH

	Batch System		Tesla Microreactor	
Treatment	Cell Concentration ($\times 10^6$ cells/mL)	Lipid Content (%)	Cell Concentration ($\times 10^6$ cells/mL)	Lipid Content (%)
Control	4.45	8.13	3.55	7.21
Alginic Acid NP	5.35	8.85	5.7	13.4
Starch NP	3.95	5.95	4.7	8.93

COMPUTATIONAL FLOW DYNAMICS (CFD)

- **Cell viability is highly affected by shear stress**
- **Average shear stress computed in COMSOL is 0.048 Pa**
- **From literature dangerous operating window is about 1 to 1.3 Pa**

$$\tau_w = \mu \left(\frac{du}{dy} \right)$$



OPTIMIZATION

MULTI-RESPONSE OPTIMIZATION

3 Factors BBD

Nanoparticles Concentration

Light Intensity

F/2 Medium Concentration

$$N = 2k(k - 1) + C0$$

Variable	Symbol	Level 1	Level 2	Level 3
NP Concentration (ppm)	A	100	300	500
Light Intensity (Lux)	B	3000	5000	7000
F/2 Media (mL/L)	C	0.5	1.25	2

REGRESSION MODEL

Cell concentration predicted model:

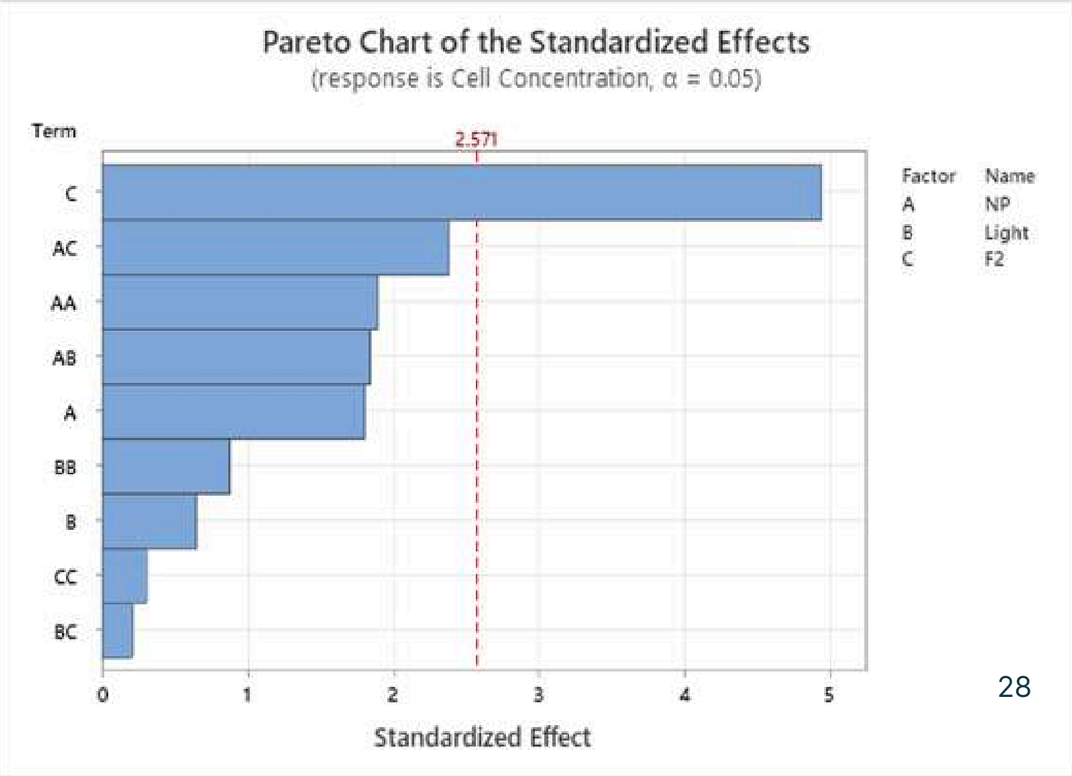
$$Ycell = 8.36\times10^{6} - 27740A + 212B + 268519C + 20.7A^{2} - 0.096B^{2} - 240741C^{2} + 1.94AB + 6667AC + 58BC$$

R^2 = 89.37% , R^2 (adj) = 70.23%

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	2.96813E+13	3.29792E+12	4.67	0.052
Linear	3	1.98694E+13	6.62313E+12	9.38	0.017
NP	1	2.31125E+12	2.31125E+12	3.27	0.130
Light	1	3.00312E+11	3.00312E+11	0.43	0.543
F2	1	1.72578E+13	1.72578E+13	24.44	0.004
Square	3	3.37879E+12	1.12626E+12	1.59	0.302
NP*NP	1	2.52579E+12	2.52579E+12	3.58	0.117
Light*Light	1	5.48478E+11	5.48478E+11	0.78	0.419
F2*F2	1	67708333333	67708333333	0.10	0.769
2-Way Interaction	3	6.43313E+12	2.14438E+12	3.04	0.131
NP*Light	1	2.40250E+12	2.40250E+12	3.40	0.124
NP*F2	1	4.00000E+12	4.00000E+12	5.66	0.063
Light*F2	1	30625000000	30625000000	0.04	0.843
Error	5	3.53104E+12	7.06208E+11		
Lack-of-Fit	3	2.72938E+12	9.09792E+11	2.27	0.320
Pure Error	2	8.01667E+11	4.00833E+11		
Total	14	3.32123E+13			

Nutrient concentration main factor affecting cell growth



SIGNIFICANT FACTORS

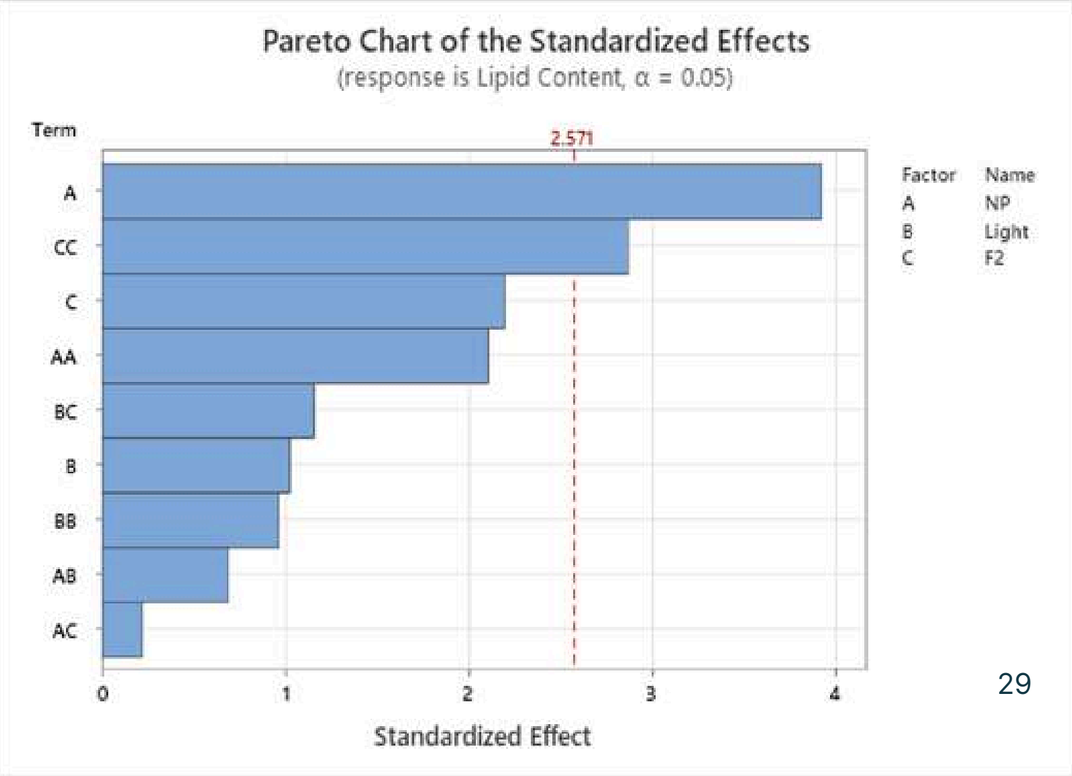
Lipid content predicted model:

$$Ylipid = -33.4 + 0.0791A + 0.006B + 27.1C - 0.000076A^2 - 7.32C^2 - 0.0000002AB - 0.00206AC - 0.001068BC$$

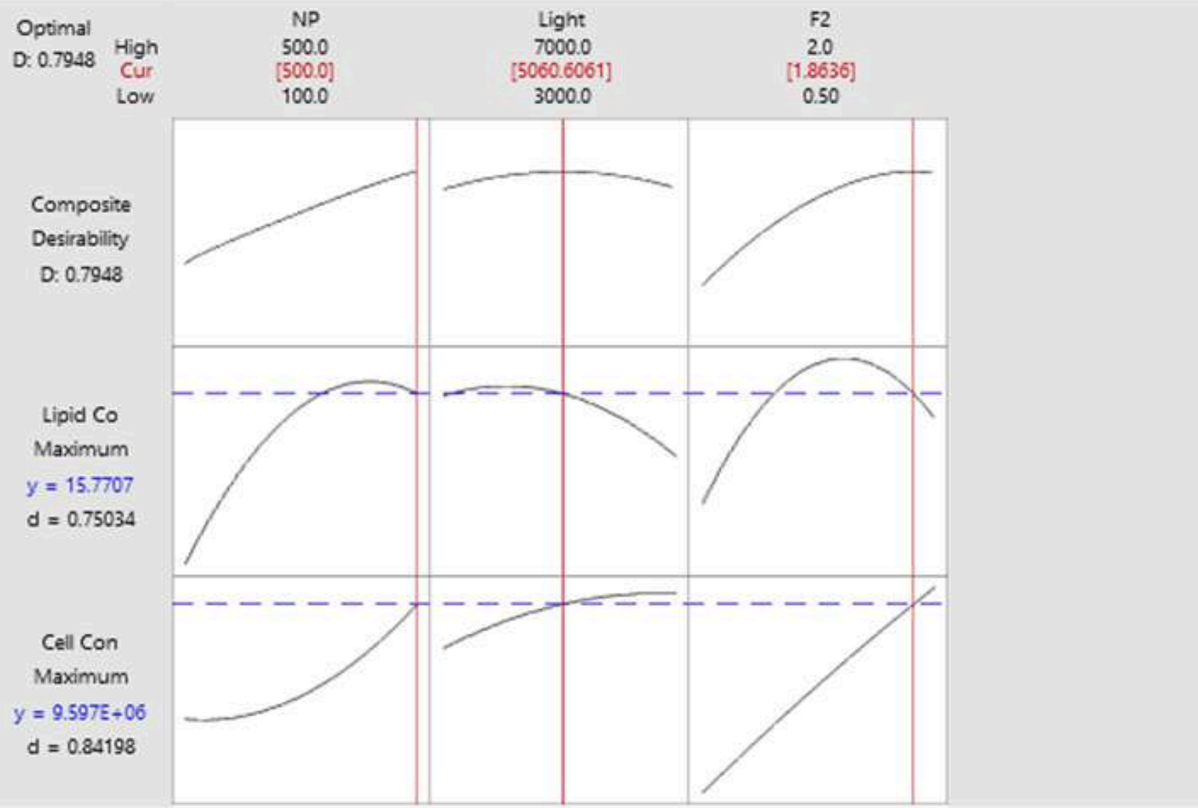
$R^2 = 87.61\%$, R^2 (adj) = 65.32%

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	268.828	29.870	3.93	0.073
Linear	3	161.897	53.966	7.10	0.030
NP	1	117.201	117.201	15.42	0.011
Light	1	8.034	8.034	1.06	0.351
F2	1	36.661	36.661	4.82	0.079
Square	3	92.644	30.881	4.06	0.083
NP*NP	1	33.851	33.851	4.45	0.089
Light*Light	1	7.026	7.026	0.92	0.380
F2*F2	1	62.518	62.518	8.22	0.035
2-Way Interaction	3	14.288	4.763	0.63	0.628
NP*Light	1	3.650	3.650	0.48	0.519
NP*F2	1	0.381	0.381	0.05	0.832
Light*F2	1	10.257	10.257	1.35	0.298
Error	5	38.007	7.601		
Lack-of-Fit	3	19.564	6.521	0.71	0.631
Pure Error	2	18.443	9.221		
Total	14	306.835			



MULTI-RESPONSE OPTIMIZER



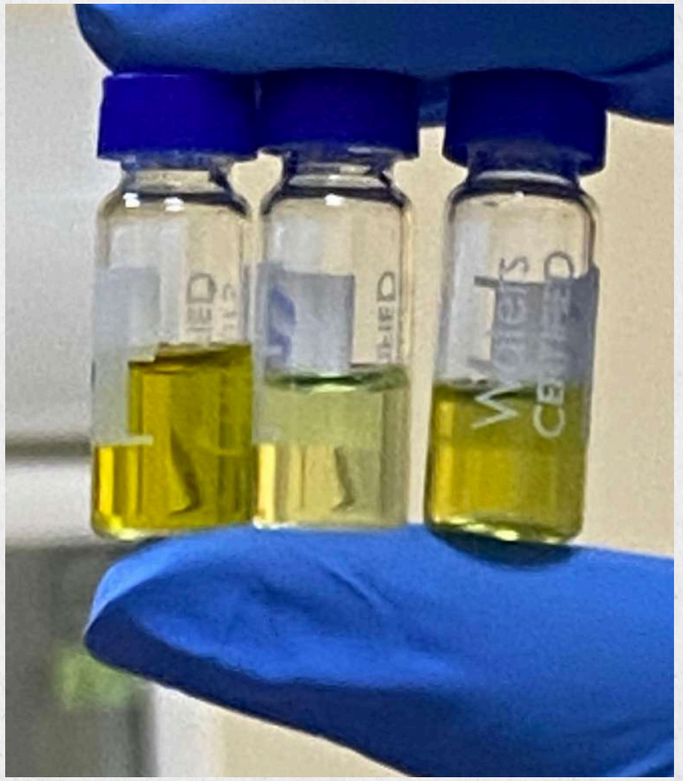
NP Concentration (ppm)	Light Intensity (Lux)	F/2 Concentration (mL/L)	Cell Density (cells/mL)	Lipid %
500	5060.6	1.8636	9.6 x 10^6	15.77

- High alginate NP concentration
- Moderate light intensity
- Moderate to high nutrient concentration

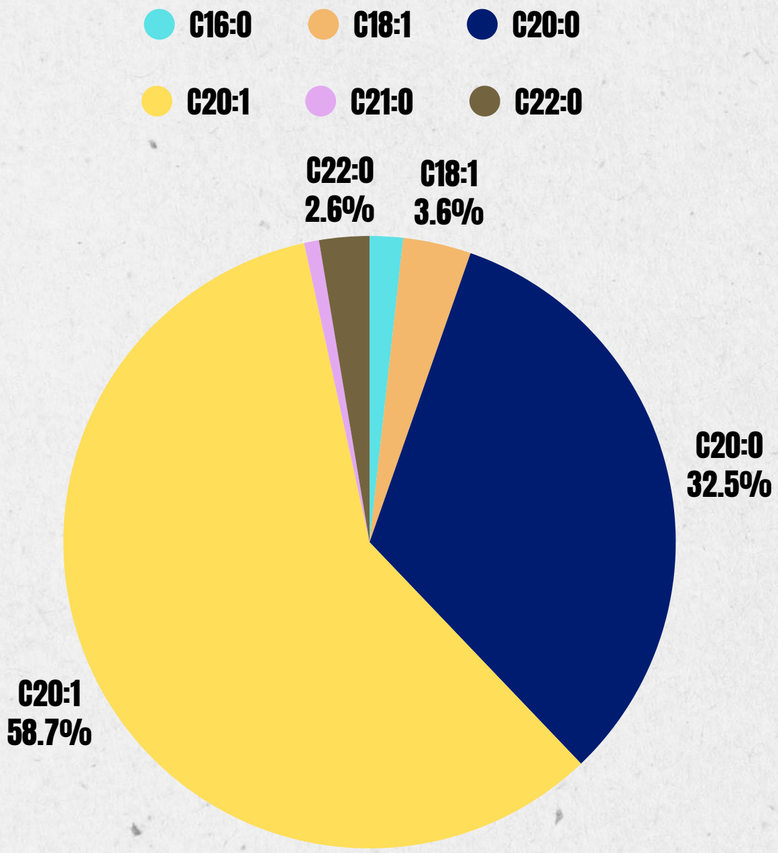
BIODIESEL PRODUCTION

- Lipids were converted into biodiesel using methanolic transesterification
- Methanol reacts with extracted lipids to produce FAME
- GC-MS was used to identify the FAME composition

$$TAG + 3CH_3OH \rightarrow 3FAME + Glycerol$$



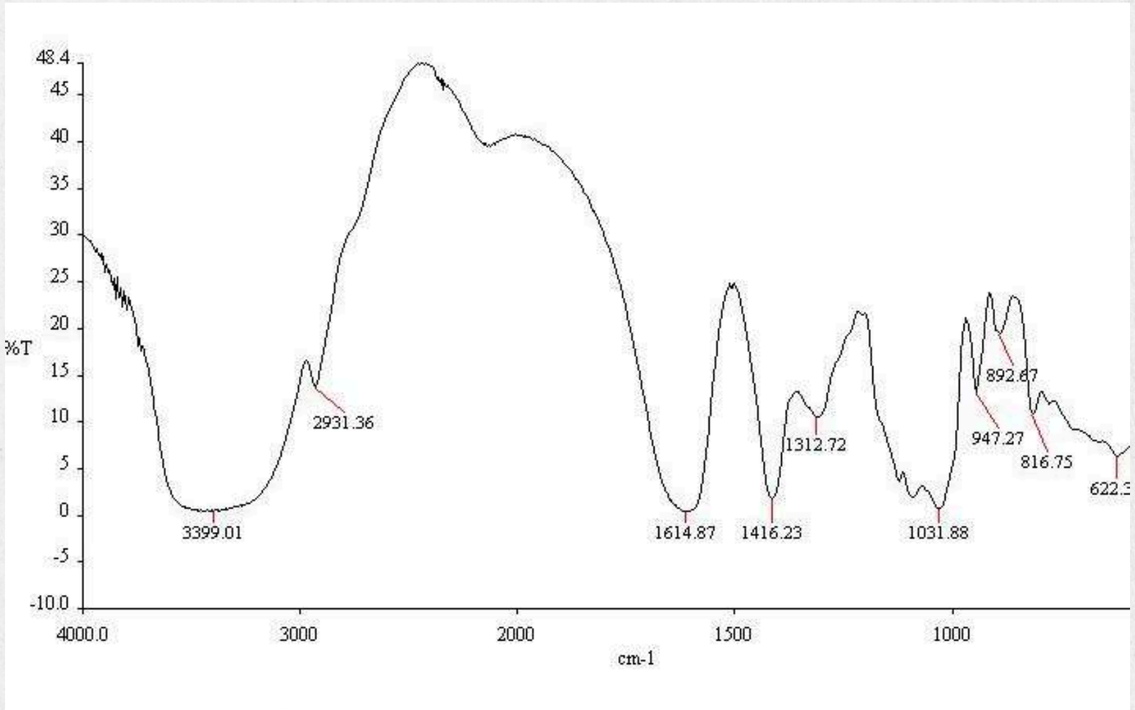
BIODIESEL ANALYSIS



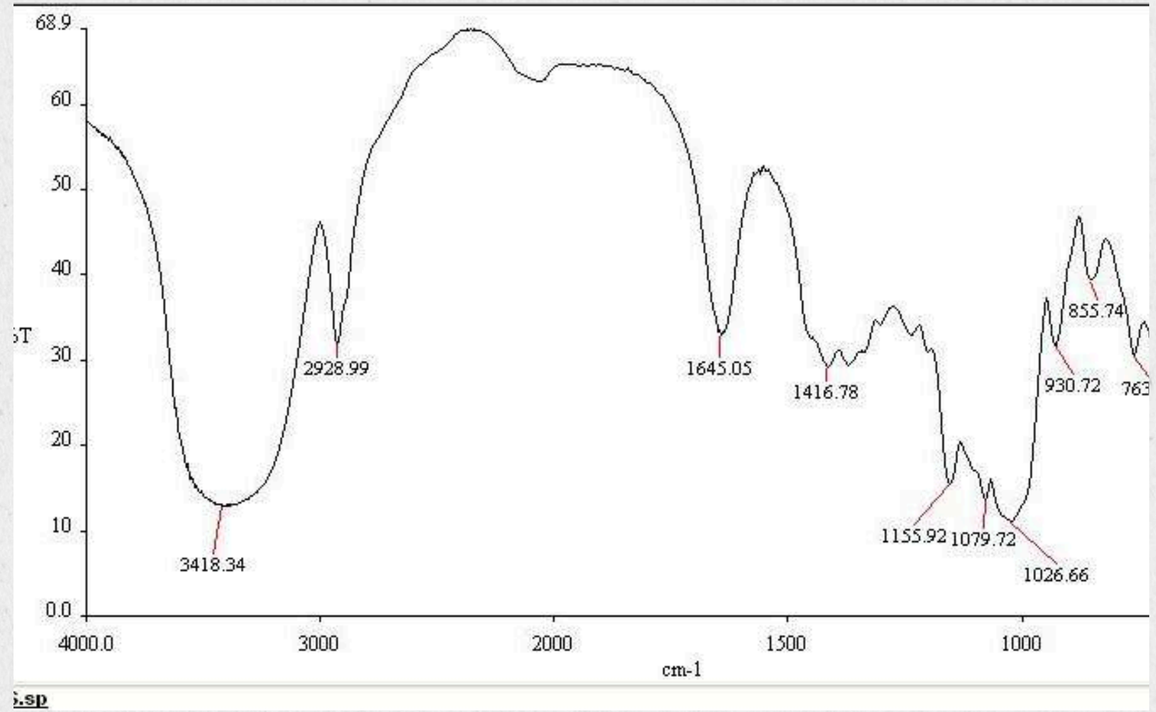
- FAME composition was analyzed using GC–MS
- Major fatty acid components were C20:1 and C20:0
- Total FAME purity reached 97.04%
- High FAME purity indicates efficient transesterification and biodiesel quality

Nanoparticles characterization (FTIR)

Alginic Acid: hydroxyl and carboxyl groups



Starch: hydroxyl and polysaccharide groups



CONCLUSION

1. Tesla microreactor with alginic NP cultivation increased *Picochlorum sp*. lipid production by 50% from batch and reached lipid productivity of 0.35 g/L/day

2. Alginic acid nanoparticle concentration was the most significant factor affecting lipid production

3. Converted lipid to FAMEs (97%)

4. Novel integration of biodegradable nanoparticles with Tesla microreactor cultivation

CONSTRAINTS

⚙️ Limited Microreactor Fabrication Availability

Difficulty finding suitable facilities for precise microreactor chip fabrication

🌊 Pump Flowrate Limitations

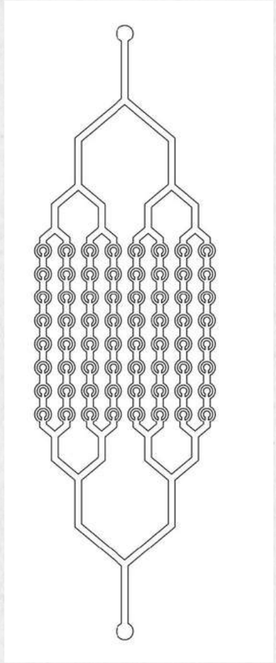
Available pumps operated at relatively high flowrates, making stable low-flow cultivation difficult

🧫 Long Experimental Duration

Microalgae cultivation and lipid accumulation required extended experimental periods

🔒 Limited Laboratory Access

Laboratory work was temporarily interrupted due to external circumstances, delaying experimental progress



FUTURE WORK

1. Use of lower flowrate pumping systems
2. Optimization of microreactor operating conditions
3. Parallel microreactor configuration
4. Scale-up studies
5. Characterization of Nanoparticles

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Dr. Muhammad Azeem

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THANK YOU !